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Addition of meloxicam to the treatment of clinical mastitis improves subsequent reproductive performance

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ABSTRACT

A blinded, negative controlled, randomized intervention study was undertaken to test the hypothesis that addition of meloxicam, a nonsteroidal anti-inflammatory drug, to antimicrobial treatment of mild to moderate clinical mastitis would improve fertility and reduce the risk of removal from the herd. Cows (n = 509) from 61 herds in 8 regions (sites) in 6 European countries were enrolled. Following herd-owner diagnosis of mild to moderate clinical mastitis within the first 120 d of lactation in a single gland, the rectal temperature, milk appearance, and California Mastitis Test score were assessed. Cows were randomly assigned within each site to be treated either with meloxicam or a placebo (control). All cows were additionally treated with 1 to 4 intramammary infusions of cephalexin and kanamycin at 24-h intervals. Prior to treatment and at 14 and 21 d posttreatment, milk samples were collected for bacteriology and somatic cell count. Cows were bred by artificial insemination and pregnancy status was subsequently defined. General estimating equations were used to determine the effect of treatment (meloxicam versus control) on bacteriological cure, somatic cell count, the probability of being inseminated by 21 d after the voluntary waiting period, the probability of conception to first artificial insemination, the number of artificial insemination/conception, the probability of pregnancy by 120 or 200 d postcalving, and the risk of removal by 300 d after treatment. Cox's proportional hazards models were used to test the effect of treatment on the calving to first insemination and calving to conception intervals. Groups did not differ in terms of age, clot score, California Mastitis Test score, rectal temperature, number of antimicrobial treatments given or bacteria present at the time of enrollment, but cows treated with meloxicam had greater days in milk at enrollment. Cows treated with meloxicam had a higher bacteriological cure proportion than those treated with the placebo [0.66 (standard error = 0.04) versus 0.50 (standard error = 0.06), respectively], although the proportion of glands from which no bacteria were isolated posttreatment did not differ between groups. No difference was observed in the somatic cell count between groups pre- or posttreatment. The proportion of cows that underwent artificial insemination by 21 d after the voluntary waiting period was unaffected by treatment. Treatment with meloxicam was associated with a higher proportion of cows conceiving to their first artificial insemination (0.31 versus 0.21), and a higher proportion of meloxicam-treated cows were pregnant by 120 d after calving (0.40 versus 0.31). The number of artificial inseminations required to achieve conception was lower in the meloxicam compared with control cows (2.43 versus 2.92). No difference was observed between groups in the proportion of cows pregnant by 200 d after calving or in the proportion of cows that were culled, died, or sold by 300 d after calving (17% versus 21% for meloxicam versus control, respectively). It was concluded that use of meloxicam, in conjunction with antimicrobial therapy, for mild to moderate cases of clinical mastitis, resulted in a higher probability of bacteriological cure, an increased probability of concep-

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tion to first artificial insemination, fewer artificial inseminations, and a greater proportion of cows pregnant by 120 d in milk.

Key words: nonsteroidal anti-inflammatory drug, mastitis, cure, fertility

INTRODUCTION

Mastitis is a very common disease in dairy herds worldwide and incurs significant costs associated with treatment, production loss, and withdrawal of milk. Epidemiological studies have shown that mastitis has negative effects on reproductive performance, including a longer interval from calving to conception, more services per conception (Barker et al., 1998; Schrick et al., 2001), lower conception rates (Santos et al., 2004; Lavon et al., 2011), and a higher risk of embryo loss (Chebel et al., 2004; McDougall et al., 2005). Effects were found for clinical mastitis caused by both gram-positive and gram-negative pathogens; however, the effect size seems to be larger for gram-negative pathogens (Hertl et al., 2010).

The timing of the mastitis event relative to insemination appears to be important. For example, conception rates to first service were 29% for cows never diagnosed with clinical mastitis, 22% for cows diagnosed before AI, 10% for cows diagnosed after AI, and 38% for cows diagnosed following confirmation of pregnancy, respectively (Santos et al., 2004). Similarly, the odds ratios for conception were 0.88 for cows diagnosed with clinical mastitis 31 to 60 d before AI, 0.81 for cows diagnosed $\leq$30 d before AI, and 0.48 for cows diagnosed within 21 d after AI, with only the latter group being different from unaffected cows (Loeffler et al., 1999a). Clinical mastitis reduces the hazard ratio (**HR**) of conception when calving to conception interval is modeled using survival analysis (HR = 0.65, 95% CI = 0.50–0.84; Suriyasathaporn et al., 1998).

Another effect of clinical mastitis is the higher risk of premature removal (culling or death) from the herd. Cows diagnosed with clinical mastitis are 1.3 to 1.5 times more likely to be removed prematurely than cows not diagnosed with clinical mastitis (Beaudeau et al., 1995; Heuer et al., 1999; Santos et al., 2004). The hazard of culling associated with mastitis increases the later in lactation that mastitis is diagnosed, with the hazard ranging from 0.5 in early through to 2.5 in late lactation (Gröhn et al., 1997; Rajala-Schultz and Gröhn, 1999a), and the culling risk is also associated with the pathogen causing clinical mastitis (Gröhn et al., 2005).

Gram-negative infections release LPS (Bannerman et al., 2004), and both gram-positive and gram-negative infections increase the concentrations of IL-1 and tumor necrosis factor α, which induce production of the enzyme cyclooxygenase (**COX**; Schmitz et al., 2004). Cyclooxygenase converts arachidonic acid into eicosanoids including prostaglandins, prostacyclins, and thromboxanes, which act as inflammatory mediators (Smith, 2005; Vangroenweghe et al., 2005). Cyclooxygenase has 2 isoforms designated as COX-1 and COX-2. The COX-1 is mainly constitutively expressed and maintains gastrointestinal mucosal integrity, aids platelet aggregation, and enhances renal blood flow. The COX-2 isoform is inducible and is upregulated in response to inflammatory stimuli. Given the increases in pro-inflammatory eicosanoids during mastitis, use of anti-inflammatory therapy may be indicated to relieve the local and systemic clinical signs of inflammation and therefore hasten return to normal physiological function of the udder (Vangroenweghe et al., 2005; Suojala et al., 2013). Nonsteroidal anti-inflammatory drugs (**NSAID**) possess anti-inflammatory, antipyretic, and analgesic properties (Smith, 2005). The NSAID may be nonselective, or selectively inhibit COX-1 or COX-2. Meloxicam is a preferential COX-2 inhibitor (Van Hecken et al., 2000; Lees et al., 2004) that has been demonstrated to reduce milk concentrations of thromboxane B_2 during mastitis (Banting et al., 2000), to reduce the plasma concentrations of the $PGF_{2\alpha}$ metabolite PGFM in endotoxin-challenged cattle (Königsson et al., 2002), and to ameliorate clinical signs of mastitis in dairy cattle (Fitzpatrick et al., 2013). Thus, NSAID may ameliorate the negative effects of mastitis on reproduction via reduced $PGF_{2\alpha}$ concentrations or via effects on gonadotrophin release, steroidogenesis, or both (Hockett et al., 2000, 2005; Lavon et al., 2011). A recent review of severe *Escherichia coli* mastitis recommends that NSAID be routinely used to supportive treatment in these cases (Suojala et al., 2013). Given that recommendation, the current study focused on mild to moderate mastitis. A recent field study on such cases has demonstrated that addition of meloxicam to antibiotic therapy resulted in a lower SCC and a lower culling proportion, particularly associated with failure to conceive (McDougall et al., 2009). Although reproductive outcomes were not specifically measured in that study, it was hypothesized that the NSAID may have been operating via reduction of negative effects of mammary inflammation on the reproductive tract.

The primary objective of the study was to assess if the combination of meloxicam and antibiotic therapy of mild to moderate cases of clinical mastitis diagnosed in the first 120 d of lactation would improve cow fertility compared with antibiotic therapy alone. Secondary objectives included assessment of the effect of addition of meloxicam on bacteriological cure and on the risk of removal within 300 d of calving.

MATERIALS AND METHODS

The study was conducted using 509 cows sourced from 61 herds consisting of a total population of approximately 7,800 cows, across 10 sites in 6 countries (Belgium, France, Italy, Spain, the Netherlands, and United Kingdom). Sites were selected on a convenience basis, with the investigator at that site agreeing to follow the protocol and to collect the required data. The investigator was asked to recruit up to 120 clinical cases from at least 3 farms. Herds were selected on the basis of being willing to follow the protocol, agreeing to allow access to cow level data, undertaking routine cow-level SCC assessment, and agreeing to allow collection of milk samples from enrolled cows. Additionally, herds were required to consist of at least 50 lactating cows and to milk twice daily. Robotic milking systems were excluded.

Herd owners were asked to present cows with signs of clinical mastitis (heat or swelling) in a single gland. Cows were excluded if they had been treated with antimicrobials or NSAID in the preceding 14 d, had previously been enrolled in the study, had a SCC >200,000 cells/mL at the 2 most recent DHIA tests in the current lactation, or had already been diagnosed pregnant in the current lactation. Only cows less than 120 d of lactation were selected because a priori it was believed likely that the majority of cows would have had at least one breeding and be potentially pregnant after this time. The investigators examined eligible cows and excluded those with very severe teat-end lesions (Mein et al., 2001) or severe mastitis [pyrexia (>40.0°C), gross evidence of dehydration or recumbency]. A California Mastitis Test (**CMT**) was undertaken of the affected gland (scored as 0, trace, 1, 2, or 3), and the presence of clots in the milk scored on a none (0), flecks (1), or clots (2) scale. Following aseptic teat end preparation and discard of the first 3 strippings, a single milk sample (~10 mL) was collected by the investigator from the affected gland for microbiology and SCC. Samples were held on ice for transport between the farm and investigators facilities for a maximum of 8 h before being stored at −20°C until sent by courier to the laboratory (GD, Deventer, the Netherlands) monthly for processing.

Cows were then treated with either meloxicam (0.5 mg/kg or 2.5 mL/100 kg s.c.; Metacam 20 mg/kg solution for injection, Boehringer Ingelheim Animal Health, Germany; treatment) or the placebo (control), which was the vehicle for the meloxicam formulation and was visually indistinguishable from the treatment. A treatment list was provided for each site, which consisted of a sequential list of numbers that correlated with a series of uniquely numbered, single-dose vials containing either the treatment or placebo. The list was generated by assigning at random within sequential pairs of numbers either the treatment or placebo. The investigator (veterinarian attending the cow) used the next sequentially numbered vial to treat the next enrolled cow. The investigators, laboratory, and analysts were blinded to the identity of the treatments until after analysis.

Additionally, all enrolled quarters were infused with a combination of 200 mg of cefalexin monohydrate and 133 mg (100,000 IU) of kanamycin monosulfate (Ubrolexin, Boehringer Ingelheim Animal Health) on 1 to 4 occasions at 24-h intervals. Milk was discarded during treatment and for 10 milkings after the final treatment.

Single milk samples (~10 mL) were collected by the investigator or trained technician from all enrolled quarters for microbiology and SCC at 14 (±3) and 21 (±3) d after initiation of treatment. The presence of clots in the milk (on a 0, 1, or 2 scale) was also recorded at these times. Herd owners were asked to record any subsequent disease diagnoses and treatments and to provide dates and reasons for culling, deaths, or sale of cows.

Herd owners were able to re-treat clinical cases where they perceived that the treatment had not resulted in clinical improvement of the affected gland. Treatment of other conditions with antibiotics or other products was allowed if required. Data concerning any treatments for any conditions were recorded for the rest of the lactation.

Cows were bred and pregnancy tested as per normal herd practice and the data retrieved for analysis. All breeding dates for enrolled cows were recorded electronically or on paper-based recording systems (or both) on farm. Pregnancy testing occurred between 26 and 270 d after insemination, and the pregnancy status (yes or no) and days pregnant were determined.

Prior to study initiation, approval from the Animal Ethics Committee of each country (where required) was sought and granted.

Microbiology

Upon receipt at the laboratory, the milk samples were stored at −20°C until processing. Processing occurred on average 2.5 d (SD = 0.4; range = 1 to 15) after receipt at the laboratory. Samples were then thawed and 10 μL 5-phase streaked over an entire 6% sheep blood agar plate and incubated for 48 h at 37°C. At 24 and 48 h, the presence or absence of colonies, the colony morphology and number of colonies were assessed. Prototheca, yeasts, and molds were identified by visual assessment of colony morphology and microscopic examination of a wet preparation at 400 times magnification. From quarter samples with a SCC >100,000

cells/mL and with ≤3 colony types at 24 or 48 h (if no growth seen at 24 h), representative colonies of each type were analyzed by matrix-assisted laser desorption/ionization-time of flight (**MALDI-TOF**) mass spectrometry (Seng et al., 2009; Bizzini and Greub 2010). When the result of initial culture was not definitive (no growth or growth of ≤10 cfu of "non-majors," or growth of *Corynebacterium* spp. only) and the SCC was ≥100,000 cells/mL (or the milk was visually abnormal), the milk sample was incubated at 37°C and was 5-phase streaked onto a 6% sheep blood agar plate and assessed after 21 ± 3 h at 37°C. A total of 0.4, 1.4, and 2.2% of samples had SCC <100,000 cells/mL and any growth at d 0, 14, and 21, respectively, and hence the growth was diagnosed on visual assessment and Gram stain. Where >3 colony types were present, double hemolytic colonies were assumed to be *Staphylococcus aureus* (n = 22 isolates; 3 pretretment and 19 posttreatment), otherwise the sample was defined as contaminated. Colonies visually assessed as *Corynebacterium* species were not tested further. Where the quarter-level SCC was <200,000 cells/mL, any isolates were identified only by visual observation. Where the MALDI-TOF result was unclear (i.e., no reliable identification or no peaks found) the identification was repeated, and if the result was not definitive, a Gram stain was performed and the result reported as a gram-positive cocci, (n = 1), a gram-negative rod (n = 2), or a gram-positive rod (n = 2).

Bacteriological cure was defined as no isolation of the pathogen(s) (identified to the genus or species level) that were isolated pretreatment in either of the posttreatment samples. So, for example, if *E. coli* had been isolated pretreatment and was not isolated in either of the posttreatment samples, the gland was defined as cured. A different bacterial genus or species may have been isolated at the posttreatment samples, but this did not affect the cure diagnosis. A new infection was defined as occurring where a gland previously defined as not infected (i.e., the gland was not defined infected at enrollment) was defined as infected at one or both time points after enrollment, or a different species or genus of bacteria was isolated from any posttreatment sample from that isolated pretreatment. Hence, it was feasible for a gland to be both cured and to have a new infection. For this reason, the status for each gland was independently defined as cured/not cured and no new infection/new infection. So to reflect the overall outcome for a gland, the final status for each gland was defined as no growth (i.e., both posttreatment samples were no growth), still infected (i.e., bacteria were isolated from one or both posttreatment samples (even where the other sample was missing, contaminated, or no growth), or not able to be determined (i.e., both samples posttreatment were contaminated or missing or where one sample was missing or contaminated and the other was a no growth). For analysis of bacteriological cure, new infection rate and final status, cure was defined at the lowest taxonomic level for which data were available. For example, where the pathogen isolated at the time of treatment was isolated to the species level, the posttreatment assessment of status was done at the same level. Conversely, where identification was only to the genus level (for example, where the isolate was defined as a CNS pretreatment), the same definition was applied for the posttreatment analyses. Note the comparison of distribution of bacteria between treatment groups was undertaken at the broader group categorization of no growth, *Staphylococcus aureus*, CNS, streptococci, gram-negative rods, and others.

Data Handling and Analysis

At the time of enrollment the cows' identity, age, clinical data, and previous SCC data in the current lactation (if any) were recorded into a purpose-built database on a tablet. At each visit to the farm, AI dates, pregnancy test results, or additional treatment data were entered into the database. The date and 1 or 2 reasons for each removal (i.e., death, cull, or sale) were recorded. Error trapping routines were included on the tablet database such that any out-of-range dates, ages, rectal temperatures, and so on could not be entered. Data from the tablet were transmitted to centralized database when the tablet had a Wi-Fi connection to the World Wide Web. Data were verified and any duplicate data excluded centrally.

The experimental unit for the study was the quarter (for bacteriological cure proportion) and the cow for fertility and removal outcomes.

The balance of treatments for age, calving date, and breed were compared using χ^2 (for categorical variables) or 1-way ANOVA (for continuous variables).

Fertility outcomes of interest were defined as follows:

- Proportion of cows bred within 21 d of the voluntary waiting period: number of cows with an insemination within 21 d of voluntary waiting period/total number of enrolled cows. The voluntary wait period was the farmer-defined interval between calving and earliest time point at which a cow detected in estrus would be bred. Where a cow was bred before the voluntary waiting period, it was included in the numerator.
- Interestrus interval: intervals (d) between the first and second AI for cows having at least 2 AI.
- Calving to first insemination: the interval in days from calving to first AI.

- Proportion of cows conceiving to first (or second and so on) AI: number of cows confirmed pregnant following the first (or second and so on) AI/total number of cows receiving a first (or second and so on) AI.
- Services per animal or services per conception: sum of number of AI/total number of enrolled cows (or number of cows confirmed pregnant).
- Proportion of cows pregnant by 120 (or 200) days after calving: number of cows confirmed pregnant by d 120 (or d 200)/total number of enrolled cows.
- Final proportion of cows pregnant (overall pregnancy rate): number of cows confirmed pregnant/total number of enrolled cows − cows with no AI − cows not pregnancy tested >30 d after last recorded AI.
- Calving to conception: the interval in days from calving to conception.

The main predictor variable was treatment (i.e., treatment versus control). However, several other variables that were potential confounders were also evaluated. These variables included age (categorized into quartiles; <4, 4, 5, >5 yr old), site, number of intramammary antibiotic treatments (1 to 4), CMT score at enrollment (coded as 0 + trace, 1, 2, 3), clot score at enrollment (0, 1, 2), rectal temperature (categorized into 1°C strata), bacteria species or genus present at enrollment (categorized as no growth, *Staphylococcus aureus*, CNS, streptococci, gram-negative rods, and others), bacteriological cure following treatment (yes/no), DIM at enrollment (categorized into 30-d blocks), days between enrollment and first service (categorized into 30-d blocks), and season of the year (i.e., January to March, April to June, July to September, and October to December).

Descriptive analyses were performed to check for outlying values and inconsistencies. Initially, the bivariate associations between the outcome variables of interest (as above) and the predictor variables were examined using χ^2 analysis for categorical variables and logistic regression for continuous variables. Those variables associated (i.e., $P < 0.2$) were then offered to a reverse stepwise procedure using a generalized linear model with a logit binomial structure and using likelihood ratio as the inclusion/exclusion criteria. Variables were removed if nonsignificant ($P > 0.05$), if they did not improve the goodness of fit as assessed using Akaike's information criterion, or if the coefficient for the treatment effect did not alter by >10%. Those variables in these models and treatment (by design) were then included in general estimating equation (**GEE**) models with cow and herd nested within site to account for the clustered nature of the data. The estimated marginal means for treatment and other main effects were calculated and the levels compared on a pairwise basis using the Bonferroni adjustment for multiple comparisons. First-order interactions between treatment and the other significant main effects were examined and included in the final model where $P < 0.05$. To assess the suitability of the GEE models, the coefficients and *P*-values from the model of conception proportion to first insemination with treatment and age group as main effects were compared with those from a binary logistic regression model (i.e., not accounting for cow with herd or herd within regions clustering) and 2- (i.e., cow within herd) and 3- (cow within herd within region) level models in STATA (V14.1, College Station, TX). In all models, treatment was significant ($P < 0.05$) and the estimate of differences in proportion of first service conception rate was highly consistent (0.099, 0.102, 0.104, and 0.109 higher for treatment than control cows in the GEE, binary logistic, and 2- and 3-level models, respectively). For speed of computation reasons, the GEE models were used. The intraclass correlations were calculated from null 2- and 3-level models to assess clustering. The intraclass correlations were calculated to be 0.05 and 0.03 in the 2- and 3-level models, respectively. This low intraclass correlation indicates very little clustering and that most of the variation is occurring between cows and not between herds or regions.

The number of AI for all enrolled cows irrespective of final pregnancy status and the number of AI required for those cows confirmed pregnant were tested using the nonparametric independent samples median test.

Survival analysis techniques were used to investigate the effect of treatment on days from calving to first insemination, from calving to conception, and from calving to removal and from initiation of treatment to next antibiotic treatment (irrespective of indication). If herd owners chose not to breed a cow, these cows were included in the analyses as the analysis was on an intention to treat basis. Cows were censored at 300 d after calving if they had failed to be inseminated or to conceive and had not been removed. For the additional antibiotic usage analysis, cows were censored at 30 d postinitiation of treatment if they had not been re-treated with antibiotics (for any indication) within that time frame. Cows removed for other reasons (e.g., death, culling) within this time frame were censored at time for removal.

For the final removal analysis, a cow was defined as removed where the herd owner had provided a date of removal and categorized the removal as a death, cull, or sale. Where no record of removal, death, or culling

was provided for a cow, the cows' ongoing presence in the herd was verified by investigators from electronic records, by phone call, or by on-farm verification of the cows' presence. Some cows (n = 23) were not able to be verified as being present. In these cases, the last data point available for that cow was used as the right censoring date. Data examined to determine the last date a cow was present included AI dates, pregnancy test dates, any additional treatment event dates, or subsequent calving date. The interval from calving (and treatment) to the last date verified was calculated for every cow. Cows were right censored at either 300 d (if the cow was not removed) or earlier for those cows that were coded as having the event of interest (removal) <300 d.

Initially, Kaplan-Meier analyses were undertaken and variables associated (Breslow tests at levels of $P < 0.2$) were included in the multivariable modeling. Daily hazard of conception (or removal) was estimated using a Cox's proportional hazards model. Model building was in a forward manual step-wise manner, with variables retained in the model when likelihood ratio statistic was $P \leq 0.05$, or when a change of >15% in the coefficient of the treatment variable occurred. The data are presented as the mean and median days from calving to first breeding (or conception; from the Kaplan-Meier analyses) and graphically as the cumulative survival proportion by days after calving (or treatment). Additionally, the HR from the final Cox's proportional hazards models are presented. An HR of >1 indicates an increased probability of the event (e.g., pregnancy or removal) occurring.

The quarter-level SCC data were natural log-transformed and effect of treatment on SCC at each time point (i.e., d 0, 14, and 21) and the change in SCC from d 14 to 0 and d 21 to 0 analyzed by one-way ANOVA. In all the cases, the data were homogenous (i.e., Levene's test for homogeneity $P > 0.15$ for all comparisons). Removals to 300 d after treatment were analyzed at bivariate level (χ^2) only. All analyses were undertaken in IBM SPSS Statistics version 22 (Chicago, IL).

Power Estimates

The study was designed to have sufficient power to demonstrate superiority of the treatment group over the control group in terms of first AI conception rate and culling proportion.

Thus, this was a superiority study with

$$h_0 = \mu_{IVP} = \mu_{CP};\ h_A\ \mu_{IVP} > \mu_{CP},$$

where μ = the proportion of cows conceiving to first AI (or culled) in the treatment (μ_{IVP}) and control (μ_{CP}) groups, respectively, h_0 = the null hypothesis, and h_A = alternative hypothesis.

The reported first-service conception rates for cows in Europe vary between 36% and 56% (Loeffler et al., 1999b; Klaas et al., 2004; Tenhagen et al., 2004; von Krueger and Heuwieser, 2010). The unweighted mean of these studies was 46%. Loeffler et al. (1999a) estimated that cows with mastitis had a 12% decline in first service conception rate compared with those without mastitis (i.e., 34% versus 46%). Similarly, Santos et al. (2004) found a 7% decline in first service conception rate where clinical mastitis was diagnosed before first insemination compared with no history of mastitis. We initially hypothesized that meloxicam would halve the loss in conception rate associated with clinical mastitis; hence the absolute difference in first service conception rate would be of order of 6 to 9.5%. If we assumed that the first service conception rate of a cow with clinical mastitis not treated with meloxicam was 30% and the first service conception rate of clinical cases treated with meloxicam was 40%, then we needed 280 cases per treatment group (assuming $\alpha = 0.05$, $\beta = 0.2$, and a 1-sided test).

In an earlier study in New Zealand assessing the effect of meloxicam on removal (culling) rates (McDougall et al., 2009), the removal percentages were 16 and 28% (odds ratio = 0.42) for meloxicam-treated and control cows, respectively. Culling rates appear to be higher in Europe (e.g., 31% overall reported by Rajala-Schultz and Gröhn, 1999b) relative to New Zealand (where approximately 20% of cows/yr are removed), and the relative risk of culling of cows with a history of clinical mastitis is about 1.5- to 2-fold higher than for nonmastitic cows. Thus, if we assumed the culling rates for cows without mastitis to be 25%, then 37% to 50% of mastitic cows were expected to be culled. If we assumed the culling rate of clinical mastitis cases without meloxicam treatment was 35% and that the odds ratio of culling was 0.7 (i.e., an absolute culling rate of 24%) with meloxicam, then we needed 220 cows per treatment group (assuming $\alpha = 0.05$, $\beta = 0.2$, and a 1-sided test).

RESULTS

Cows Included

Five hundred and nine cows were initially enrolled into the study. No reproductive (AI dates, pregnancy test data, and so on) or additional treatment data were

provided for 4 cows at one site, and these cows were excluded from the reproductive analyses. Additionally, 3 cows were excluded from the reproductive analyses because they had been confirmed pregnant before enrollment date and incorrectly enrolled. Thus, 502 cases were eligible for the reproductive analyses.

Group Balance

Treatment groups did not differ by allocation within site, age of cow, CMT, or clot scores at enrollment, bacterial species at enrollment, or the number of intramammary treatments given (all $P > 0.1$; Table 1). The rectal temperature at the time of enrollment also did not differ between treatment groups [38.6°C (SD = 0.5) versus 38.7°C (SD = 0.5) for treatment versus control cows, respectively; $P = 0.48$]. Cows in the meloxicam group were enrolled on average 8 d later in lactation than the placebo group [49.7 d (SD = 35.8) versus 41.5 d (SD = 35.2) for treatment versus control cows, respectively; $P = 0.01$].

Additional Treatments after Enrollment

A total of 89/502 cows were treated with an additional antibiotic within 30 d of initiation of primary treatment. The number of glands and cows re-treated did not vary between treatment groups [41/250 (16.4%) versus 48/252 (19.0%) for treatment versus control, respectively, $P = 0.44$].

A total of 6 cows (5 from the meloxicam group, 1 from the control group) were culled (n = 3; one each for mastitis, production, and lameness) or died (n = 3) <30 d after initiation of treatment and were censored on the day of death or culling.

Bacteriology

No difference was found in the distribution of bacteriological groups between treatment groups at the time of enrollment (Table 1).

At the bivariate level, bacteriological cure was associated ($P < 0.2$) with treatment group [80/118 (67.8%)

Table 1. Number (no.) and percentage (within treatment) of cows by treatment group for cows with mild to moderate mastitis treated with intramammary antibiotics and parenterally with either meloxicam or placebo[1]

		Meloxicam		Placebo		
Item	Category	No.	%	No.	%	*P*-value
Site	1	11	4.3	11	4.3	1.00
	2	28	11.1	28	10.9	
	3	8	3.2	9	3.5	
	4	2	0.8	2	0.8	
	5	11	4.3	11	4.3	
	6	60	23.7	60	23.4	
	7	19	7.5	20	7.8	
	8	55	21.7	55	21.5	
	9	19	7.5	20	7.8	
	10	40	15.8	40	15.6	
Age (yr)	<4	97	39.4	97	38.3	0.16
	4	57	23.2	49	19.4	
	5	44	17.9	37	14.6	
	>5	48	19.5	70	27.7	
California Mastitis Test score d 0	0 + trace	6	2.4	8	3.1	0.82
	1	56	22.1	63	24.6	
	2	112	44.3	112	43.8	
	3	79	31.2	73	28.5	
Clot score d 0	0	53	20.9	58	22.7	0.82
	1	121	47.8	124	48.4	
	2	79	31.2	74	28.9	
Bacteria d 0	No growth	69	31.7	73	32.3	0.26
	Staphylococcus aureus	9	4.1	11	4.9	
	CNS	24	11.0	32	14.2	
	Streptococci	49	22.5	50	22.1	
	Gram-negative rod[2]	34	15.6	25	11.1	
	Others	23	10.6	15	6.6	
	Mixed	10	4.6	20	8.8	
No. of intramammary tubes	1	3	1.2	7	2.7	0.53
	2	183	72.3	190	74.2	
	3	21	8.3	19	7.4	
	4	46	18.2	40	15.6	

[1]The *P*-values are derived from the bivariate analyses.

[2]*Citrobacter* species, *Enterobacter* species, *Escherichia coli*, *Klebsiella* species, *Pasteurella* species, *Pseudomonas* species, *Serratia* species.

Table 2. Outcome of glands infected at the time of diagnosis of clinical mastitis by treatment group for cows with mild to moderate mastitis treated with intramammary antibiotics and parenterally with either meloxicam or placebo[1]

	Cure				New infection			
	Meloxicam		Placebo		Meloxicam		Placebo	
Species/genus at enrollment	No	Yes	No	Yes	No	Yes	No	Yes
Bacillus species	2	2		2	3	2	1	1
Citrobacter species		1			1	1		
CNS	2	3	1	5	2	3	4	2
Corynebacterium species		1		2	1		2	
Enterobacter cloacae		1				1		
Enterobacter species				1			1	
Enterococcus cecorum								
Enterococcus species	1	1	1	1	1	1	2	1
Escherichia coli	2	17	2	17	10	10	9	11
Klebsiella pneumoniae				1				1
2 bacterial species[2]	4	5	8	8	5	4	9	5
Mixed flora[3]					4	8	4	3
Mold								1
Pasteurella multocida	1					1		
Proteus species		1				1		
Prototheca species	1	2	2		1	2		1
Pseudomonas species				1			1	
Serratia marcescens	1	6	1		2	6	1	
Staphylococcus aureus	8	1	7	1	7	2	7	
Staphylococcus chromogenes		5	1	4		5	4	3
Staphylococcus haemolyticus		5	1	3	5		2	2
Staphylococcus sciuri		3		5	1	2	2	3
Staphylococcus simulans			2				2	
Streptococcus dysgalactiae ssp. *dysgalactiae*	3	5	2	8	4	4	2	7
Streptococcus species Lancefield-group G	3	3	5	3	5	2	4	3
Streptococcus uberis	7	16	15	5	11	16	11	10
Trueperella pyogenes			2				1	
Viridens streptococci			1				1	
Yeast	3	2	1		3	1		1
No growth					24	29	23	36
Total	38	80	52	67	90	101	93	91

[1]Cure was defined as not growing the bacteria present pretreatment at either of the posttreatment sampling time points. Where 2 bacterial species were grown, cure was defined where neither species were grown at either of the posttreatment sampling points. New intramammary infection was defined as occurring either where a gland previously uninfected (no growth) subsequently isolated bacteria posttreatment, or where a different bacterial species was isolated posttreatment from that isolated pretreatment.

[2]Two bacterial species were cultured.

[3]More than 2 species were grown; that is, the sample was contaminated.

versus 67/119 (56.3%) for treatment versus control, respectively; Table 2], rectal temperature at the time of enrollment, and bacteria at the time of enrollment. Cure rate was not associated with the number of intramammary treatments, the clot or CMT score at the time enrollment, the age of the cow, DIM at treatment, or the site.

In the final GEE model, bacteriological cure proportion was associated with treatment and bacteria group. Cows treated with meloxicam had a higher bacteriological cure proportion than those treated with the control [0.66 (SE = 0.04) versus 0.50 (SE = 0.06) for treatment versus control, respectively; RR = 1.99 (95% CI 1.17–3.40); $P = 0.012$]. Glands infected with *Staphylococcus aureus* had a lower bacteriological cure proportion than glands infected with other species or groups. Gram-negative rods and CNS had better cure proportion than other pathogens. No interaction was found of treatment by bacteria ($P = 0.56$).

A total of 198/382 (51.8%) of glands acquired a new intramammary infection. At the bivariate level, the rate of new infection was associated ($P < 0.2$) with site, clot score at enrollment, bacterial species at enrollment, cure rate, age, DIM at enrollment, and season. As cure rate was counfounded with treatment group, bacterial group at enrollment was used in the final model. In the final model, the new infection tended to be higher in cows treated with meloxicam than the control group [0.51 (95% CI 0.40 to 0.62) versus 0.44 (95% CI = 0.37–0.52), for treatment versus control, respectively; $P = 0.08$]. New infection rates increased with age ($P < 0.001$), were highest in April to June ($P < 0.001$), and were lowest in glands infected with *S. aureus* at enrollment ($P = 0.001$).

The final bacteriological status was determined for 397 of 509 (80%) glands. No difference was found either at bivariate or multivariate level in proportion of glands that did not have an intramammary infection posttreatment (i.e., were pathogen free at both posttreatment samplings) by treatment [56/189 (29.6%) versus 61/208 (29.3%) for treatment versus control; $P = 0.97$]. The only variable associated with the gland being pathogen free following treatment was whether the gland had cured [0.32 (SE = 0.04) for bacteriologically cured glands versus 0.20 (SE = 0.02) for glands not cured; $P = 0.01$].

SCC Pre- and Posttreatment

There was no difference in the Ln SCC at d 0, 14, or 21 or in the difference between d 14 and 0 or d 21 and 0 for treatment versus control (all $P > 0.5$; Table 3). The Ln SCC at d 14 (5.80 ± 0.15 versus 7.78 ± 0.22 for cured versus uncured glands, respectively; $P < 0.001$) and d 21 (5.66 ± 0.16 versus 7.78 ± 0.24 for cured versus uncured glands, respectively; $P < 0.001$) were lower in those glands in which bacteriological cure occurred. No treatment group by cure interactions were found for Ln SCC at d 14 or 21, or in the difference between d 14 and 0 or d 21 and 0 (all $P > 0.05$).

Reproduction Outcomes

A summary of the fertility performance is presented in Table 4.

Proportion of Cows Bred Within 21 of the Voluntary Waiting Period. A total of 272 of the 502 cows (54.2%) received AI within 21 d of the end of the voluntary waiting period. At the bivariate level, the likelihood a cow would receive AI was associated with site, number of intramammary treatments given, and the clot score at the time of enrollment. In the final GEE model, no effect was found of treatment on the proportion of cows that received AI [0.53 (SE = 0.02) versus 0.52 (SE = 0.02) for treatment versus control, respectively; $P = 0.74$]. Sites differed in the likelihood that a cow would receive AI ($P < 0.001$).

Inter-Estrous Intervals. Fewer cows treated with meloxicam had 18- to 24-d intervals between first and second AI (Table 4; Figure 1; $P = 0.04$).

Calving to First Insemination: Survival Analysis. A total of 458 of the 502 cows (91.2%) had an AI by 300 d after calving. At the bivariate level, no difference was found between groups in the proportion of cows that had not received AI by 300 d after calving [19/249 (7.6%) versus 25/253 (9.9%) for treatment versus control, respectively]. The hazard of AI was associated with site, age category, CMT score at enrollment, diagnosis of clinical mastitis before the voluntary waiting period, and the season of the year in which diagnosis occurred.

In the final multivariate (Cox's) proportional hazards model, the hazard of AI varied among sites ($P < 0.001$). No effect was found of treatment on the hazard of first AI [HR 0.95 (95% CI = 0.79–1.14), $P = 0.55$; Figure 2].

Proportion of Cows Conceiving to First AI. A total of 126 of the 442 cows (28.5%) conceived to the first AI. Conception rate to first AI was associated ($P < 0.2$) at the bivariate level with site, treatment, age category, clot score at enrollment, and number of intramammary treatments. Days in milk at the time of treatment, DIM at first service, and interval between

Table 3. The natural log (Ln) of SCC (×1,000 cells/mL) for quarters diagnosed with mild to moderate clinical mastitis and treated parenterally either with meloxicam or control

						95% CI	
Item	Treatment	No.	Mean	SD	SE	Low	High
Ln SCC d 0	Meloxicam	144	8.02	1.69	0.14	7.74	8.30
	Control	152	8.03	1.68	0.14	7.76	8.30
	Total	296	8.03	1.68	0.10	7.83	8.22
Ln SCC d 14	Meloxicam	195	5.94	1.98	0.14	5.66	6.22
	Control	205	6.03	2.10	0.15	5.74	6.32
	Total	400	5.99	2.04	0.10	5.79	6.19
Ln SCC d 21	Meloxicam	194	5.94	2.12	0.15	5.64	6.24
	Control	203	5.90	2.19	0.15	5.59	6.20
	Total	397	5.92	2.15	0.11	5.70	6.13
Ln SCC d 14 to 0[1]	Meloxicam	124	−2.12	2.16	0.19	−2.51	−1.74
	Control	134	−2.18	2.21	0.19	−2.55	−1.80
	Total	258	−2.15	2.18	0.14	−2.42	−1.88
Ln SCC d 21 to 0[1]	Meloxicam	117	−2.21	2.31	0.21	−2.63	−1.79
	Control	132	−2.35	2.35	0.20	−2.75	−1.95
	Total	249	−2.28	2.33	0.15	−2.57	−1.99

[1]Difference in Ln SCC on d 14 (or d 21) and d 0.

Table 4. Number of cows (No.+ve) and proportion (Propn) of cows or median and 95% confidence intervals for dichotomous and continuous measures of reproductive performance of cows with mild to moderate clinical mastitis treated with intramammary antibiotics and parenterally with either meloxicam or placebo (control)[1]

	Meloxicam			Control			
Parameter	No. +ve	Total	Propn	No. +ve	Total	Propn	*P*-value
Dichotomous measure							
Bred by 3 wk VWP[2]	136	249	0.55	136	253	0.54	0.85
Proportion returns 18 to 24 d[3]	37	144	0.26	62	170	0.36	0.04
Conceive to AI #1	75	225	0.33	51	217	0.24	0.02
Conceive to AI #2	46	144	0.32	47	170	0.28	0.41
Conceive to AI #3	31	89	0.35	29	114	0.25	0.15
Pregnant by d 120	104	249	0.42	84	253	0.33	0.05
Pregnant by d 200	161	249	0.65	151	253	0.60	0.25
Pregnant final	192	224	0.86	189	216	0.88	0.58
Continuous measure							
	Meloxicam			Control			
		95% CI			95% CI		
Interval (d)	Median	Low	High	Median	Low	High	
Calve to first AI	77	73	81	78	75	81	0.45
Calve to conception	132	122	142	154	136	172	0.26

[1]The *P*-values are from χ^2 (dichotomous) or Kaplan-Meier (continuous) univariable analyses.

[2]Voluntary waiting period.

[3]Proportion of cows with an inter-estrus interval of 18 to 24 d/all cows with an inter-estrus interval.

treatment and first service were not predictors of first AI conception proportion.

In the final GEE model, treatment with meloxicam was associated with an increased first AI conception proportion compared with the control [0.31 (SE = 0.02) versus 0.21 (SE = 0.02) for treatment versus control; RR = 1.68 (95% CI = 1.25–2.25); $P < 0.01$]. First service AI proportion was lower in 5-yr-old and >5-yr-old cows compared with <4-yr-old cows ($P < 0.01$), and the conception proportion varied by site ($P < 0.001$).

Number of Inseminations. Over all cows, cows in the meloxicam group received fewer AI than those

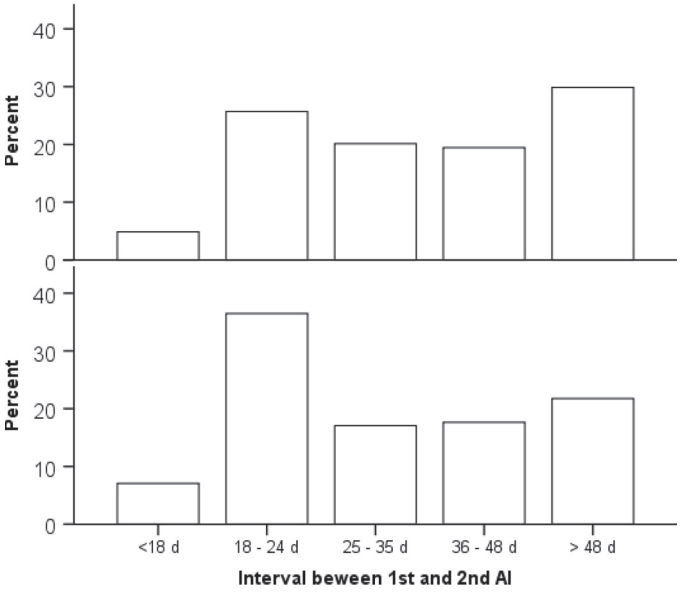

Figure 1. The interval (d) between the first and second AI by treatment group: meloxicam (top) or control (bottom).

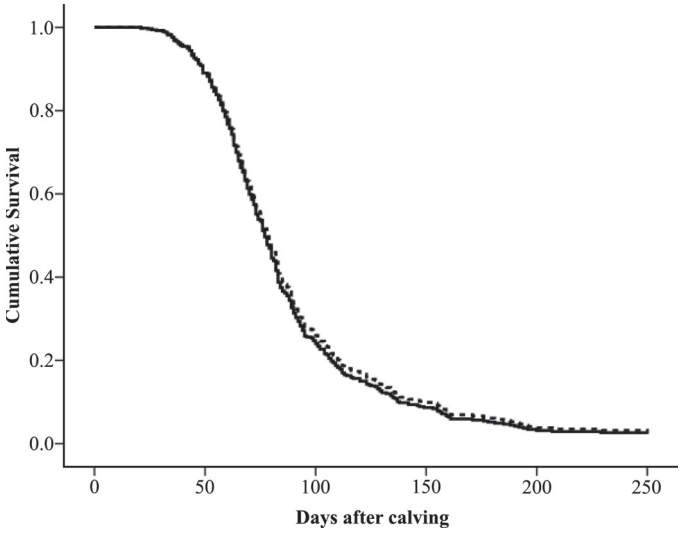

Figure 2. The cumulative survival (i.e., not receiving AI) for cows diagnosed with mild to moderate clinical mastitis treated with intramammary antibiotics and parenterally with either meloxicam (solid line) or control (dashed line).

in the control group [2.46 (95% CI = 2.20–2.72; median = 2) versus 2.70 (95% CI 2.45–2.96; median = 2) for treatment versus control, respectively; $P = 0.04$]. Among those cows confirmed pregnant, the number of AI required to achieve conception was lower among those cows treated with meloxicam compared with the untreated controls [2.43 (95% CI = 2.17–2.69; median = 2) versus 2.92 (95% CI 2.65–3.19; median = 2) for treatment versus control, respectively; $P = 0.009$].

Proportion of Cows Pregnant by 120 d After Calving. A total of 188 of 502 (37.5%) of cows were confirmed pregnant by 120 d after calving. At the bivariate level, the proportion of cows pregnant by 120 d was associated ($P < 0.2$) with treatment, rectal temperature at enrollment, age group, and whether the cow had been diagnosed with clinical mastitis before or after first service.

In the final GEE model, more treatment than control cows were pregnant by 120 d after calving [0.40 (SE = 0.01) versus 0.31 (SE = 0.01); $P < 0.001$], and the proportion pregnant varied among sites ($P < 0.001$) and among age groups ($P < 0.001$).

Proportion of Cows Pregnant by 200 d After Calving. A total of 312 of 502 (62.2%) of cows were confirmed pregnant by 200 d after calving. At the bivariate level, the proportion of cows pregnant by 200 d was associated ($P < 0.2$) with age group, whether the cow had been diagnosed with clinical mastitis before or after first insemination and season of year in which diagnosis occurred.

In the final GEE model, no difference was found between treatments in proportion of cows pregnant by 200 d after calving [0.61 (SE = 0.03) versus 0.56 (SE = 0.02); $P = 0.14$], but the proportion pregnant varied among age groups ($P < 0.001$) and season of the year in which enrollment occurred ($P < 0.001$) with a lower proportion in April to June and higher proportion in July to September compared with October to December.

Final Proportion of Cows Pregnant (Overall Pregnancy Rate). A total of 62 cows were either not bred at all (n = 44), did not have any pregnancy diagnosis undertaken (n = 16), or did not have pregnancy diagnosis undertaken after the last recorded insemination (n = 2). A tendency was found for less of the meloxicam than control cows to have an unknown final pregnancy status [25/249 (0.10) versus 37/253 (0.15) for treatment versus control, respectively; $P = 0.12$]. No difference was found among groups in the proportion of cows finally defined as pregnant [192/224 (0.86) versus 189/216 (0.88) for treatment versus control, respectively; $P = 0.58$].

Calving to Conception Interval. At the bivariate (Kaplan-Meier) level, the hazard of pregnancy was associated ($P < 0.2$) with bacteria group at enrollment, age category, timing of diagnosis and treatment relative to AI, and season in which diagnosis occurred. The median interval from calving to conception was 132 (SE = 5) versus 154 (SE = 9) d for the treatment versus control, respectively ($P = 0.26$).

Hazard of Pregnancy. In the final Cox's proportional hazards model, the hazard of pregnancy tended [HR = 1.21 (95% CI 0.98–1.50); $P = 0.08$] to be higher for treatment than control cows (Figure 3a). Cows <4 yr old conceived more quickly than cows 5 or >5 yr old ($P < 0.001$; Figure 3b). Cows diagnosed with mastitis before the first insemination were slower to conceive than those diagnosed after first insemination ($P < 0.001$; Figure 3c). Site was included in the model but was not significant ($P = 0.42$).

Removals. A total of 158 of 505 (31.3%) cows were recorded as having died, been culled, or been removed during the study. The mean day of removal was 294 d after calving (median = 283 d; SD = 160 d; range = 5 to 704 d) and 82 (51.9%) and 76 (48.1%) of the 158 cows were removed <300 and ≥300 d after calving, respectively.

At the bivariate level, no difference was found in the proportion of cows removed, died, or sold <300 d of treatment between the treatment groups (Table 5; $P = 0.97$). Additionally, the reasons provided for removal did not differ among those cows culled <300 d after treatment (Table 6; $P = 0.84$).

Survival to 300 d Posttreatment. The survival to 300 d after treatment did not differ among treatment groups at the bivariate level (Figure 4; $P = 0.24$). Survival was affected by age ($P < 0.01$) and the clot score at the time of enrollment ($P = 0.03$).

In the final Cox's proportional hazards model, treatment was not associated with risk of removal [HR = 1.22 (95% CI = 0.82–1.83), $P = 0.32$], whereas younger cows (i.e., <4 yr old) were less likely to be removed than older cows [HR = 2.25 (95% CI = 1.24–4.10), HR = 3.12 (95% CI = 1.71–5.68), and HR = 2.79 (95% CI = 1.60–4.88) for 4-, 5-, and >5-yr-old cows, respectively, relative to <4-yr-old cows; $P < 0.001$].

DISCUSSION

This study demonstrated that addition of meloxicam to antimicrobial treatment of mild to moderate clinical mastitis resulted in an increased first AI conception proportion and an increased proportion of cows pregnant by 120 d after calving. Additionally, at the univariable level, meloxicam-treated cows had fewer 18 to 24 d returns and fewer inseminations were needed to achieve conception. The hazard of pregnancy tended to be higher for cows treated with meloxicam.

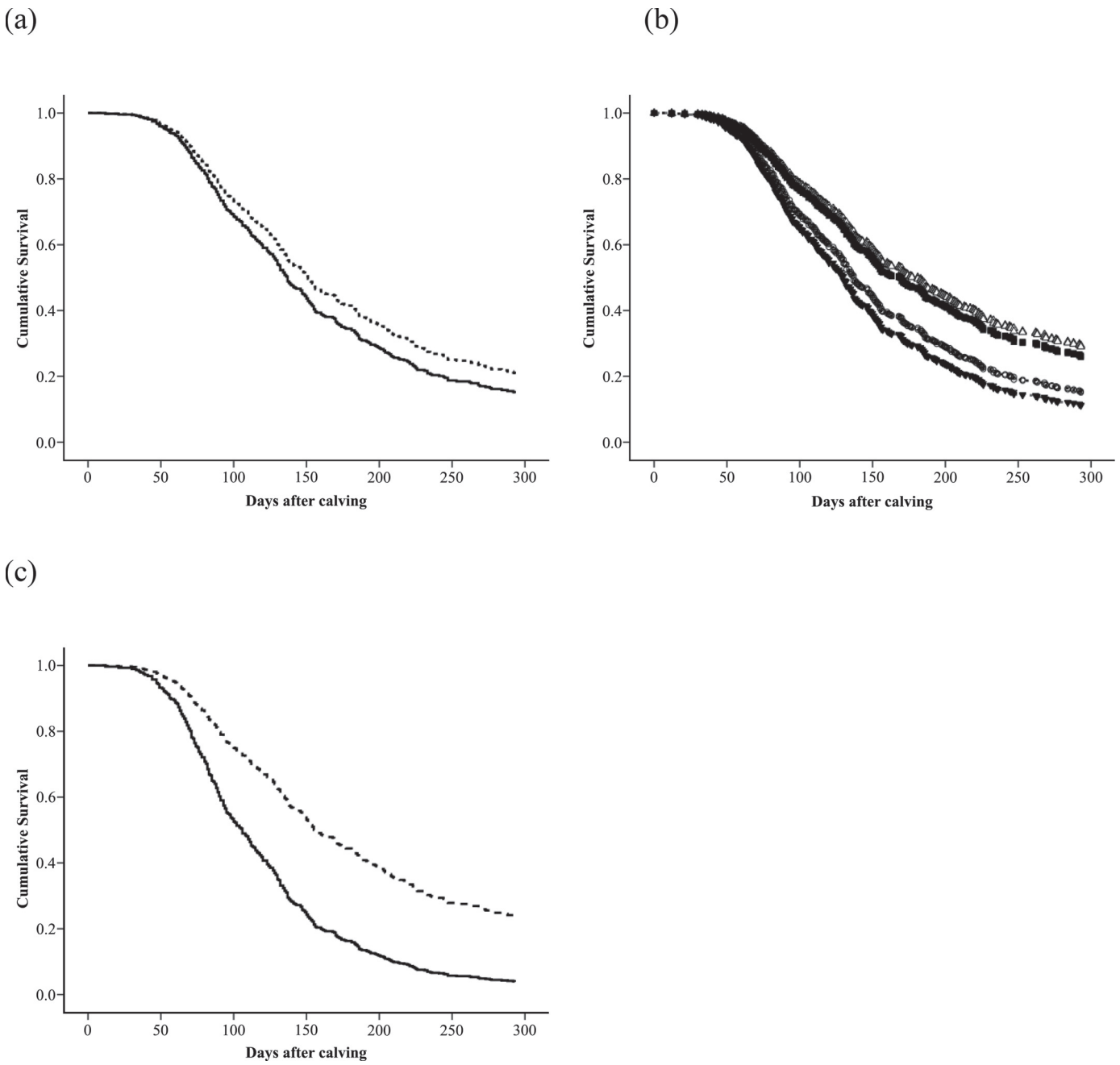

Figure 3. Cumulative survival (i.e., not pregnant) of cows with mild to moderate mastitis treated with intramammary antibiotics and parenterally with either with meloxicam or placebo by (a) treatment group (meloxicam = solid line; control = dashed line), (b) age group (<4 = ▼; 4 = ○, 5 = Δ, >5 = ■), and (c) diagnosis and treatment of clinical mastitis before (solid line) or after first AI (dashed line).

This study was undertaken across 8 regions in 6 countries across Europe. As such, it is likely to have a high level of external validity. Randomization of cows to treatment was undertaken at region (site) rather than herd level. This randomization approach was used because it was believed that only a small numbers of cases would be enrolled in any individual herd and the time over which it was likely take to enroll cases would be such that the variability between herd within a region within a short time period (a few weeks) may be less than the variability within herd across seasons and across an extended period of time. Due to the hierarchal nature of the data (i.e., cow within herd within region), it is feasible that an unequal allocation of treatment and controls across herds differing in reproductive performance could potentially result in

Table 5. Number (no.) and proportion (Propn) of cows listed as culled, died, or sold within 300 d after treatment of cows with mild to moderate mastitis treated with intramammary antibiotics and parenterally with either meloxicam or placebo

	Meloxicam		Control		Total
Item	No.	Propn	No.	Propn	No.
Not removed	215	0.86	208	0.82	423
Culled	29	0.12	39	0.15	68
Died	2	0.01	2	0.01	4
Sold	5	0.02	5	0.02	10
Total	251		254		

bias, such that effects ascribed to treatment may in fact be due to herd level effects. In a study of reproductive performance of across 3,207 lactations in 1,570 herds, cows in 50 herds in 5 geographic regions, it was found that 86% of the variation in reproductive performance was at the lactation level, with only 7, 6, and 2% of the variability of cow, herd, and regional levels, respectively (Dohoo et al., 2001). The current study also found low intraclass correlations, suggesting that the great majority of variation was occurring between animals and not between herds and regions. Thus, even if by chance allocation was unequal of treatment and control to herds of differing overall reproductive performance, the effects of the cow are more important in determining outcome than the herd or regional level effects. Further support for this was found in that the coefficients for the effect sizes were very similar whether clustering was accounted for or not. That is, models that did or did not account for the hierarchical nature of the data provided very similar estimates of first service conception proportion. If significant confounders were present at herd or regional level, it may have been expected that the significance or effect sizes, or both, associated with treatment would be variable among these models. So whereas herd level management factors may influence reproductive performance and hence increase the variability in the outcome measures, this variability may be no more than the between lactation, cow, or herd variability over a short period. At a region level, the herds were clients of one veterinary business, and hence advice, programs, and procedures would likely be consistent for reproductive management across these groups of herds. Taken together, these data suggest that although allocation bias may have occurred due to the experimental design, the likelihood that this would have resulted in errors of inference (that is, falsely concluding the presence of a treatment effect when in reality one was not present, or vice versa) or biases in the estimates of the size of the treatment effect appears low.

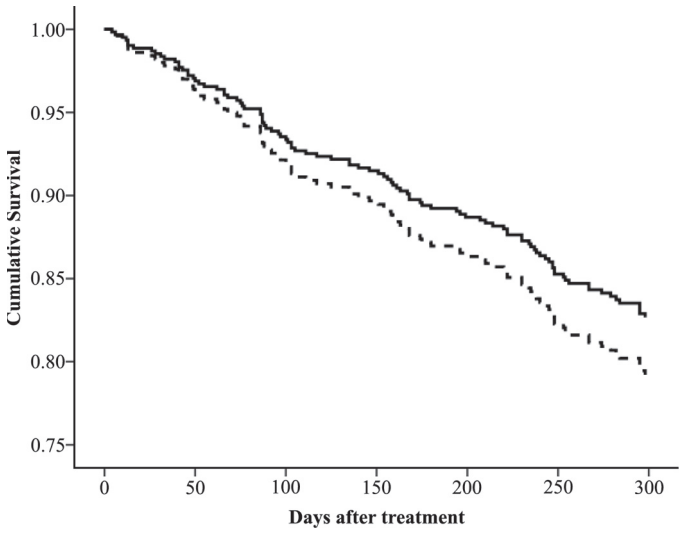

Figure 4. Cumulative survival (i.e., not died, culled, sold) for cows with mild to moderate clinical mastitis treated with intramammary antibiotics and parenterally with either with meloxicam (solid line) or control (dashed line).

Several epidemiological studies have demonstrated that mastitis reduces conception proportion (Santos et al., 2004; Lavon et al., 2011), increases the number of services required per conception, and increases the interval from calving to conception (Barker et al., 1998; Schrick et al., 2001). Mastitis is also associated with altered inter-estrus intervals (Moore et al., 1991). The current study has demonstrated that treatment with

Table 6. The primary reason for removal of cows within 300 d after treatment of cows with mild to moderate mastitis treated with intramammary antibiotics and parenterally with either meloxicam or control

Primary reason	Meloxicam	Control	Total
Unknown	0	1	1
Low milk yield	7	7	14
Mastitis	10	12	22
Reproductive failure	9	9	18
Udder breakdown	1	3	4
Other	9	14	23
Total	36	46	82

meloxicam ameliorated some of the negative effects of mastitis on reproductive performance.

Although it has previously been demonstrated that mastitis is associated with an extended calving to first AI intervals (Barker et al., 1998; Schrick et al., 2001; Huszenicza et al., 2005) and a reduced probability of expressing estrus (Hockett et al., 2005; Lavon et al., 2011), in this study no effect was found of treatment on the interval from calving to first AI or the proportion of cows receiving AI by 21 d after the end of the voluntary waiting period. This study did not directly assess the probability of expression of estrus associated with ovulation.

The bovine mammary gland produces $PGF_{2\alpha}$ (Hansel et al., 1976) and increases in $PGF_{2\alpha}$ occur in milk from cows with clinical mastitis (Anderson et al., 1986). Challenge studies have demonstrated that LPS increases circulating concentrations of prostaglandins (including $PGF_{2\alpha}$), which shortens the lifespan of the corpus luteum (Giri et al., 1990). A similar increase in $PGF_{2\alpha}$ was found after mastitis induction with *Streptococcus uberis*, indicating that this is not only a gram-negative effect (Hockett et al., 2000). Early luteolysis or early embryo resorption may alter inter-ovulatory and hence inter-estrus intervals. Pregnancy recognition occurs approximately 16 d after insemination and is dependent on a functional corpus luteum. Hence, if mastitis were to shorten luteal lifespan, pregnancy recognition and conception have an increased likelihood of failure. The lower proportion of 18 to 24 d returns suggests that meloxicam may have a protective effect on luteal function by reducing $PGF_{2\alpha}$ concentrations.

Mastitis may also affect the hypothalamus-pituitary axis. Some cows challenged with *Streptococcus uberis* had reduced LH pulse frequency, had reduced estradiol concentrations, and did not express estrus or ovulate (Hockett et al., 2005). Those authors hypothesized that the effects of *S. uberis* on estrus expression were via inhibitory effects of inflammatory cytokines on pituitary LH release, independent of any effects on prostaglandin concentrations. Additionally, evidence indicates that mastitis affects follicular steroidogenesis, which causes delay in, or failure of, ovulation (Lavon et al., 2011). This may be mediated via cytokines as IL1-β, IL-2, TNFα, and IFNα have been shown to inhibit estradiol production by granulosa cells in vitro (Spicer and Alpizar, 1994) and direct effects of LPS on estradiol concentrations have been demonstrated (Herath et al., 2007). Thus mastitis may be having negative effects on reproduction via mechanisms independent of prostaglandin. Evidence from in vitro studies suggests that NSAID can suppress production of some pro-inflammatory cytokines including IL-1 and IL-6 (Jiang et al., 1998; Berg et al., 1999).

Clinical mastitis occurring soon after insemination has been associated with greater negative effects on reproductive performance than clinical mastitis before insemination (Santos et al., 2004). When clinical mastitis occurred any time between 14 d before, to 35 d after, AI was associated with a lower conception rate (Hertl et al., 2010), indicating that a closer temporal relationship between mastitis and AI has a greater negative effect. However, in the current study, no association was found between timing of clinical mastitis and AI conception rate, nor did the timing of diagnosis of clinical mastitis and treatment relative to the time of AI modulate the treatment effect. This conclusion was reached by including the interval treatment and insemination as a main effect and then specifically testing whether the effect of treatment varied with differing intervals between treatment and AI by testing the treatment by time interaction for the key fertility outcomes. In no case did the interaction term for treatment by time appear in a final model. Although the power to find such an interaction was limited by the study size, the inference from the current study is that the effect of meloxicam is similar irrespective of the timing of treatment relative to AI. The duration of effect of the NSAID treatment, however, appears to be longer than that associated with a reduced $PGF_{2\alpha}$ concentrations. Thus, it appears likely that some other, longer term mechanism, or an additive effect of multiple mechanisms, is operating. Chronic effects of mastitis on follicular dynamics have been demonstrated (Lavon et al., 2011), and hence if NSAID were to ameliorate the initial effects of mastitis, longer term negative effects may be reduced. This is demonstrated by the fact the treatment affected first service conception rate and the proportion of cows pregnant to d 120 postpartum, even where the interval between treatment and insemination was weeks to months in some cases. Although not significant, the treated cows had a higher proportion of cows pregnant by d 200 postpartum, but this is likely due to the faster rate of conception early postpartum.

A previous study investigating the effect of addition of meloxicam to antimicrobial therapy of mild to moderate mastitis found that inclusion of meloxicam resulted in a reduced probability of removal (McDougall et al., 2009). Mastitis has been associated with an increased risk of culling, irrespective of the stage of lactation at which it occurs (Beaudeau et al., 1995; Gröhn et al., 1997; Rajala-Schultz and Gröhn 1999a,b). The risk of culling associated with mastitis may be due to direct effects of the disease and its treatment or due to reduced production or increased risk of failing to conceive. Reproductive status is the single most important factor in culling decisions and failure to conceive at first AI or an extended interval

from calving to conception increase the risk of culling (Beaudeau et al., 1995). However, once conception has occurred, risk of culling is decreased (Gröhn et al., 1998). Across the entirety of the current study, 31% of cows were culled or died. A total of 31% of Finnish Ayrshire cows from the entire population (independent of preceding disease status) were removed each lactation (Rajala-Schultz and Gröhn, 1999a). It had been expected that clinical mastitis would increase culling rate compared with cows within mastitis in the current study, but as culling data from the rest of the population in enrolled herds was not collected, this could not be verified. The current study found a nonsignificant 4% reduction in the proportion of removals following meloxicam compared with untreated control cows in the 300 d postcalving. By this stage, only 18% of the control cows and 14% of the treatment cows had been removed. In the continuous calving systems used by all herds in the current study, almost half the cows that were removed during that lactation were removed more than 300 d after calving. Thus, the decision during the design phase of the study to right-censor removals at 300 d may have led to an underestimate of the effect of treatment on this outcome. A potential explanation for the nonsignificance of meloxicam treatment effect on removal by 300 d compared with the significant finding in the previous study (McDougall et al., 2009) is that in the latter study, which was undertaken in seasonally calving herds, those cows not conceiving in the 12- to 14-wk breeding period were removed at the end of the lactation which is commonly <300 d. In contrast, cows in the current study remained in the herds, in some cases well beyond 300 d, even if they had failed to conceive, presumably because farm owners believed these animals were still producing sufficient milk to be economical.

This appears to be the first study that has demonstrated an increased bacteriological cure rate of mild to moderate clinical mastitis cases following treatment with an NSAID. Positive benefits of NSAID in terms of resolution of inflammation associated with mastitis have been demonstrated (Vangroenweghe et al., 1995). Meloxicam may be acting by reducing the degree of udder edema and swelling (Fitzpatrick et al., 2013), potentially resulting in improved distribution of the antimicrobial in the quarter. Alternatively, its effects may be being modulated indirectly via improved host response associated with earlier return to rumination, or an increased DMI as has been previously demonstrated in some (Yeiser et al., 2012) but not all (Fitzpatrick et al., 2013) studies using NSAID treatment of mastitis.

It has previously been demonstrated that meloxicam treatment of clinical mastitis results in lower SCC at quarter level 2 to 3 wk after treatment (McDougall et al., 2009). The current study did not replicate that finding. Several explanations are possible for this. The previous study was undertaken in pasture-based systems, and the pathogen distribution was different. Additionally, in the current study the proportion of glands that were uninfected following treatment did not differ between groups. So, although meloxicam resulted in higher bacteriological cure, the higher new infection rate posttreatment resulted in the same prevalence of IMI posttreatment, resulting in no difference in SCC.

CONCLUSIONS

Use of the nonsteroidal anti-inflammatory drug meloxicam, in addition to antimicrobial therapy for treatment of mild to moderate cases of clinical mastitis, results in a higher probability of bacteriological cure, and improved fertility in terms of higher conception to first service, higher probability of pregnancy by 120 d postcalving, a reduced number of inseminations required to achieve pregnancy and a tendency to have a shorter calving to conception interval.

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